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EDITORIAL

FUNDAMENTAL KNOWLEDGE

We have referred to this subject on several occasions, and again come back to it. In this number of the *Veterinary Journal* we publish an article by J. G. Speed and P. G. D. Morris on "The Adrenals of the Horse": this article is an example of research work from the results of which our knowledge of the structure of a tissue has been improved. There is no doubt that in much of our applied work we suffer from an insufficiency of accurate information on the structure and functions of some of even the commonest tissues. Until such defects have been remedied the full significance of any apparent abnormality can be but imperfectly understood. It will be observed that in the summary of the above-mentioned article the following sentence occurs: "An attempt is made to describe the glands in animals considered to be healthy, as a basis for comparison with the adrenals of animals suffering from a grass-sickness and certain other conditions." Without a clear and reliable knowledge of the normal, it is impossible to assess the importance of the apparently abnormal. In the course of our search for the causes of some of the more obscure types of disease in livestock we may find a variety of conditions which suggest that certain processes are at fault, and that therein lies the root cause. The results of these altered processes may be reflected in an alteration of the macroscopic and/or microscopic picture of a tissue; it may be that the altered picture was the reason for the further examination of the tissue and the eventual narrowing down of the field of enquiry to the process involved. All such enquiry and work, followed by lines of reasoning, however, must be based on an established and accurate knowledge of the normal; unless normal

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structures and processes are known and understood under the varying conditions of "health," our observations may lead us along the wrong path for further enquiry, with much loss of time and the expending of effort which could be used profitably in other directions.

In order to obtain this fundamental knowledge of structure, composition and action of normal tissues or processes, somewhat lengthy research work has to be undertaken; we fear that, in some work at least, too much stress may have been laid on the results of observations on too few a number of specimens or animals, and under too restricted circumstances. We must pursue our enquiries over a sufficiently large field before we can assume that a structure or a process falls into the realm of abnormality. In the study of biochemical findings we have a good example of the normal variation of the presence of many substances in tissues in apparently healthy animals. We find, by reference to text-books and the scientific literature, that "normal values" are given for many of the elements, etc., in blood and other tissues, and it is surprising to note the wide variation that may occur. Such information must always be acquired from the examination of many tissues and animals which are known to be in a normal or healthy condition. The "border-line" findings must always give rise to concern and speculation; the practised "eye" is valuable in assessing a healthy from an unhealthy animal.

Our studies in the future, then, must be so arranged that we can differentiate the normal from the abnormal to a greater extent than in the past. Histology, in its wide sense, has an important place in our research work; there are definite indications that with the altered outlook on the causes and methods of control of disease, histology has been somewhat forgotten. It has a place, and an important one, in all our work, and, with physiology and biochemistry, must be used to the full in our endeavour to establish standards of normality, and so be able to recognise abnormality of tissues and structures. A full appreciation of the normal will go a very long way in our efforts to understand some of the complicated processes which lead to disease, and therefore loss, from so-called obscure causes.

General Articles

THE ADRENALS OF THE HORSE

By J. G. SPEED, M.R.C.V.S., and P. G. D. MORRIS, M.A., M.R.C.V.S.,
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In the course of our dissection of horses attention was drawn to the considerable variations in the appearance and weight of the adrenal glands. Bearing in mind the susceptibility of these glands to changes believed to be brought about by nutritional variations, sexual condition, and disease processes, it was decided to try to find what could be described as the normal condition of the adrenal glands in at least one breed of horses.

Material

Clydesdale horses were used, the source of supply being animals slaughtered for human food. The clinical history, age, sex, and general condition of these animals were ascertained as well as possible, and note was made of any abnormality seen in post-mortem examination. It was not possible to weigh the horses, and therefore it was decided to classify them in three groups, light, medium and heavy, i.e., animals of 11-13 cwts., 13-15 cwts. and 15 cwts. upwards.

The animals were shot, bled, carefully eviscerated, and the adrenals dissected out of the perirenal fat. It was found most convenient to have the animal lying on its back with the hind legs raised by means of a pulley and chain. The abdominal cavity was opened along the midline and the intestine pulled carefully forward. The small colon was cut through near the pelvis, and the mesocolon cut along near its attachment to the colon. The cæcum and large colon were freed by hand from their attachment to the pancreas, the mesentery and cranial mesenteric vessels were cut an inch or two from the root of the mesentery, and this permitted the intestine to be pulled forward out of the abdomen.

At this stage the ventral border of the left adrenal could usually be seen lying along the medial border of the left kidney, but only the posterior extremity of the right adrenal could be seen, lying ventral to the hilus of the right kidney and lateral to the vena cava.

The adrenals could now be dissected out, but first, search was made in the fat and connective tissue surrounding them for the appearance of accessory adrenal bodies. These may be confused on superficial examination with the small lymph nodes which are abundant in this region.

In the case of the left adrenal it was comparatively easy to free it from the perirenal fat and to cut through the vessels and nerves which enter it on its lateral face. The right adrenal presented more difficulty: the middle portion of the right adrenal lies lateral to the vena cava and is attached to it, while the anterior extremity projects into the renal impression of the liver and there occupies the space between the anterior pole of the kidney, the vena cava, and the liver. It was therefore necessary to cut away a portion of the vena cava and remove it, attached to the adrenal; frequently it was found convenient to cut away part of

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the caudate process of the liver to allow the anterior pole of the adrenal to be reached. When the adrenals had been dissected out, the fat was cleared from the capsule and the glands were first weighed and then their volume measured by immersing them in water, in a glass measuring cylinder.

An outline of both surfaces of each gland was then drawn on paper, and this outline was used to map in any nodules or irregularities of the surface of the gland. Measurements of the glands were also taken by calipers, but did not seem to have any obvious significance.

General Appearance

In colour the adrenals vary from a red-brown to a yellow-brown.

Left adrenal. The left is usually sickle-shaped, the convex curvature ventral and posterior extremity relatively blunt. Both medial and lateral surfaces are flattened.

One abnormally-shaped left adrenal was found; this occurred in a mare, the caudal extremity of the adrenal being flattened out to form a base 5.2 cm. wide, the general shape of the adrenal being triangular, with the anterior extremity forming the apex. The weight of this adrenal was in proportion to that of the right adrenal from the same animal.

Right adrenal. The right adrenal is less regularly shaped. It is usually thickest and widest at its middle, where a wide, well-marked groove for the vena cava crosses its medial surface obliquely. The anterior extremity is pointed and pyramidal, with three surfaces. Characteristically, one or two slit-like openings are present on the medial surface where the blood drains directly into the vena cava.

General Remarks

In both adrenals the surface of the capsule is smooth when the fat has been dissected off, although on the lateral or renal surface the cut ends of the vessels and nerves mark what may be called the "hilus."

Smoothness of the surface of the capsule is not a constant character, and usually one or more nodules or prominences are visible. These nodules varied so much in size and number in the different specimens examined that some attempt was made to correlate their occurrence with variations in the ages of the animals. (See Table.)

CLYDESDALES

Sex	Age	Type L=Light M=Medium H=Heavy	Weight R. gms.	Weight L. gms.	Weight Total gms.	Vol. Total ccs.	Nodules Large brown	Nodules Small yellow	Remarks
Mare	3	M	15.85	14.55	30.4	29.0	0	8	Animal destroyed because it was vicious
Mare	6	M	17.9	17.3	35.2	34.3	5	6	Unknown history. Normal P.M.
Stallion	8	H	21.02	18.55	39.6	39	18	8	Animal was not a successful stallion
Gelding	9	M	19.15	17.5	36.7	35.5	2	9	Fistulous withers
Gelding	13	L	26.1	24.8	50.9	50	12	24	Unknown history. Normal P.M.
Gelding	14	H	24.5	21.4	45.9	44.9	5	15	Unknown history. Normal P.M.

Sex	Age	Type L=Light M=Medium H=Heavy	Weight R. gms.	Weight L. gms.	Weight Total gms.	Vol. Total ccs.	Nodules Large brown	Nodules Small yellow	Remarks
Gelding	15	M	23.5	20.65	44.2	44	11	30	Normal p.m. Lame
Mare	17	M	23.6	21.5	45.1	45	3	14	Retention cysts in kidneys
Mare	20?	L	28.7	30.71	59.6	57	8	66	Normal p.m. Unthrifty condition. Old age.
				CLYDESDALES (BLIND)					
Gelding	13	M	34.5	35.9	70.4	65	4	58	Cause of blindness unknown
Mare	18	L	26.6	25.3	51.9	49.1	10	68	Ditto
				CLYDESDALES (ABNORMAL P.M.'s)					
Gelding	7	L	30.75	26.65	57.4	55.9	15 (large)	2	Active synovitis of carpal region
Mare	9	H	38.2	36.8	75	73.5	19 "cottage loaf" nodules & accessories	27	Acute nervous condition
Mare	9	H	28.5	33.7	62.2	60.5	76 mostly bosselation	3	Chronic thick leg (Lymphatic œdema)
Mare	13	M	34.1	30.2	64.3	62	14 and areas of bosselation	30	Acute lymphangitis ("weed")
Gelding	18	H	33.5	34.9	68.4	65.2	60 mostly areas of bosselation	8	Enlarged spleen and general appearance of grass sickness in P.M.
				CROSS CLYDESDALES					
Gelding	13	M	24	23	47	47	15	14	Obscure lameness. Normal P.M.
Gelding	13	M	24.8	21.5	46.3	44	5	10	Greasy legs. Normal P.M.
Gelding	16	L	15.3	14.75	30	28.9	9	10	Normal P.M. Blind
Mare	18-20	M	20.7	21.5	42.2	41	10	8	Old age. Normal P.M.

Blood Supply

An injection preparation was made, using a celoidin masse, and it was found in this case that the blood supply was carried to the gland by a number of branches given off by the renal artery.

The venous drainage of the right adrenal is by one, two, or three direct openings from the adrenal capsule through the wall of the vena cava. It was noted that these openings are guarded on the caval side by a flap or valve of the endothelial lining of the vena cava.

Accessory Adrenals and Nodules on the Main Glands

Search was made during the dissecting-out of the adrenals for accessory adrenal tissue. Several cases were found in which small discreet rounded

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nodules of adrenal tissue were present in the perirenal fat near the adrenal glands, and not in any way connected with the main gland. It was recognised that examination by naked eye and palpation were necessarily crude methods and unlikely to discover all the accessory adrenals present, but it seemed unlikely that in any of the adrenals examined the discreet accessory bodies were numerous enough to cause disproportion in the comparison of total weights of adrenal tissue.

The nodules and irregularities on the surface of the gland were quite another matter. In some cases, noticeably in young animals, in good health, the surface of the glands was almost free from irregularities. For instance, in a young mare, three years old, only two very small nodules were present on the left adrenal and three, equally small, on the right. (Fig. I.)

In another case, that of an aged mare some 18-20 years old, there were twenty-four nodules, large and small, on the left adrenal and fifty on the right. In another, a blind mare some 16-18 years old, there were forty-eight nodules on the left adrenal and thirty on the right.

These nodules vary considerably in appearance. Some are mere rounded elevations of the same colour as the gland itself, are generally large in size, and isolated from each other. The other common type are small, sharply-defined, yellow tubercles, which usually stand out more prominently from the gland and are frequently in groups or clusters.

Instances were found of nodules superimposed upon other nodules in the form of a cottage loaf, and one or two cases in which the lower component of the " cottage loaf " had a depression in its centre and the top component was absent. From this it seemed a possible conjecture that these nodules could become on some occasion detached from the main gland, and that they could form discreet accessory adrenal bodies.

The third definite type of irregularity of the surface occurred in our experience only in animals with abnormal post-mortem appearance. This took the form of large areas of what can best be described as bosselation. One animal in which it occurred had a greatly enlarged spleen, much fluid in the small intestine, the large colon impacted with hard, dry ingesta, and the carcase had a most pronounced smell.

It was decided not to include the adrenals from this animal in the collection of normal material, but to consider them as being from a pathological case. (Fig. III.)

Histology

Technique. Before making any preparations for microscopical study, free hand sections and later thick (50 μ.) frozen sections were made through portions of the glands which showed a distinct nodule or nodules on the surface. The glands were previously fixed in 10 per cent. formol. By these methods, sets of thick serial sections were obtained and examined with a dissecting microscope.

For more detailed examination, paraffin blocks from portions of the glands were made and serial sections cut through some of the nodules. The portions of the glands used for sectioning were put into a fixative as soon as possible after the completion of the macroscopical examination; this meant that the material was placed in the fixative 1-2 hours after removal from the freshly-killed animal. The

adrenal being a gland in which post-mortem changes take place very rapidly, these blocks were only used for what may be called a gross histological examination. The blocks of tissue used for examination of the cellular arrangement and more detailed histological structures were placed in fixatives within a few minutes after the destruction of the animal.

Fixatives.

(1) 10 per cent. formol.

(2) Zenker-formol.

(3) Orth's fluid.

Each of the above fixatives was used. Zenker's formol was found to be the most satisfactory, the fixation being more nearly perfect than with Orth's fluid, which appeared to cause considerable shrinkage of the tissues. Orth's fluid was used because it showed the chromaffin reaction very distinctly, but it was found that Zenker-formol showed this reaction equally well.

Stains.

(1) Hæmatoxylin and Eosin as the routine stains.

(2) Masson's Trichrome stain and modified Azan stain to demonstrate connective tissue.

(3) Verhoeff's elastic tissue stain to demonstrate the presence of elastic tissue fibres.

(4) Weddell's silver impregnation method to demonstrate nerve fibres.

(5) Wilder's silver impregnation for reticulum fibres.

(6) Sudan IV to demonstrate fat in frozen sections.

(7) Eosin methylene blue.

General Histology

Examination with a dissecting microscope of free hand and frozen sections showed that the general structure of the adrenal gland of the horse was the same as in other animals, that is to say, there were present a capsule, cortex, and medulla. The cortex appeared a bright yellowish-brown, the medulla a dark brown, showing several large sinus-like blood vessels. Large trabeculæ were visible running from the capsule down into the cortex, thus causing a lobing of the cortical tissue.

Microscopical Examination

Capsule. This is thick, consisting of white fibrous tissue containing a few elastic fibres. Lying on the surface of the capsule and embedded in it are numerous blood vessels, nerve trunks and small ganglia. From the capsule many delicate strands of connective tissue run down into the parenchyma of the gland;

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these fine connective tissue strands divide up the glandular tissue into cell groups and columns. In addition to these fine strands of connective tissue, thick trabeculæ from the capsule dip down into the substance of the gland as far as the medulla. It was noted that the Zona glomerulosa and Zona fasciculata followed the trabeculæ down into the gland substance. Many of the larger blood vessels and nerves are carried into the gland within these trabeculæ. (See Fig. XX.)

Cortex. In the horse adrenal the cortex is clearly divisible into three zones. There is a clear line of demarcation between the cortex and medulla (Figs. XII and XIII), but they are not completely separated from one another as in some animals, e.g., dog, cat, and pig (*Textbook of Histology,* Jordan), by a connective tissue capsule surrounding the medulla; there is, however, in the horse an incomplete layer of connective tissue between the cortex and medulla.

Zona Glomerulosa. The zone immediately under the capsule is the Zona glomerulosa; here the cells are arranged in arched columns (see Fig. X); in the centre of the arch there is a connective tissue core containing many capillaries (see Fig. X). The cells of the Zona glomerulosa are long columnar-shaped cells, their nuclei are oval and elongated in shape, the chromatin is scanty, three or four distinct nucleoli are present (see Fig. XI). The cytoplasm of the cells is finely granular, showing numerous fatty vacuoles at the poles of the cells. The presence of this fat is readily demonstrated by staining with Sudan IV (see Fig. XXIII). The fat is not so abundant in this zone as in the Z. fasciculata or Z. reticularis (see Fig. XXI).

Zona Fasciculata. The cells of this zone are arranged in long, straight columns, which extend from the Z. glomerulosa to the Z. reticularis. Between the columns of cells there are fine connective tissue fibres, carrying with them small capillaries and nerves; there is also a definite supporting network of reticular fibres (see Fig. XIX). The cells are polyhedral in shape with a round centrally-placed nucleus, in which the chromatin is scarce; a distinct nucleolus is present. Their cytoplasm is frequently filled with fatty vacuoles (see Figs. XIV and XXII).

Zona Reticularis. This is the innermost layer of the cortex. It is clearly separated from the medulla, but not by a complete capsule of connective tissue, as is found in some animals (dog, cat, and pig), although the fine connective tissue strands running between the cortical cells do collect together and form an incomplete capsule between the Zona reticularis and medulla.

The cells of the Z. reticularis are arranged in irregular columns with large blood sinusoids between them (see Figs. XII and XIII). The cells of this zone are smaller than those of the Z. fasciculata, polyhedral in shape, with a round centrally-placed nucleus; their cytoplasm is finely granular and acidophilic, and many of them show fatty vacuoles (see Fig. XIII). In the part of the Z. reticularis next to the medulla, here and there amongst the typical cells of the Z. reticularis, some large, somewhat oval-shaped cells were noticed. The cytoplasm of these cells stained feebly and showed many fine granules, which appeared a yellowish-green colour in an H. & E. stained section, and a bluish-green colour in a section stained with eosin and methylene blue. The nuclei of these cells often showed a shrunken and degenerate appearance.

Medulla. The medulla forms a central core to the gland. In the horse it is

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KEY TO PHOTOGRAPHS

Fig. 1.—Adrenals of 3-year-old Clydesdale mare.
A—Aortic surface of left adrenal.
B—Caval surface of right adrenal.

Fig. 2.—Left adrenals. Renal surface from Clydesdale stallion 8 years old.
A—Typical reddish-brown circumscribed nodule.

Fig. 3.—Left adrenal from 18-year-old gelding with abnormal post-mortem appearance.
A—Bosselations of the cortex.
B—Small yellow circumscribed nodules.

Figs. 4, 5, 6.
A—Main gland.
B—"Nodule" of cortical tissue.
C—Accessory Chromaffin tissue.
D—Connecting neck of cortical tissue.

Figs. 7, 8, 9.
A—Main gland.
B—"Nodule" of cortical tissue.
C—Connecting neck of cortical tissue.

Fig. 10.
A—Capsule.
B—Cells of zona glomerulosa.
C—Connective tissue core and capillaries.
D—Zona fasciculata.

Fig. 11.—High-power zona glomerulosa.

Fig. 12.
A—Medulla.
B—Zona reticularis.
C—Zona fasciculata.

Fig. 13.
A—Medulla.
B—Zona reticularis.
C—Blood sinusoids.

Fig. 14.—Cells of zona fasciculata.

Fig. 15.—Medulla, showing cells arranged in groups.

Fig. 16.
A—Medullary cells arranged in groups.
B—Columnar cells surrounding blood vessels.
C—Venous sinus.

Fig. 17.
A—Accessory chromaffin tissue.
B—Capsule.
C—Zona glomerulosa.

Fig. 18.
A—Small nerve cells in medulla.

Fig. 19.—Zona fasciculata showing reticular network.

Fig. 20.
A—Capsule.
B—Fine connective tissue septa.
C—Large fibrous trabeculæ.
D—Zona glomerulosa.

Fig. 21.—True accessory body.
A—Capsule.
B—Zona glomerulosa.
C—Zona fasciculata.
D—Zona reticularis.
E—Blood sinuses.

Fig. 22.—Frozen section stained Sudan IV.
A—Cortex (showing fat).
B—Medulla.

Fig. 23.—Zona glomerulosa.
A—Fatty droplets (stained Sudan IV).

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FIG. 1. Three-quarters actual size.

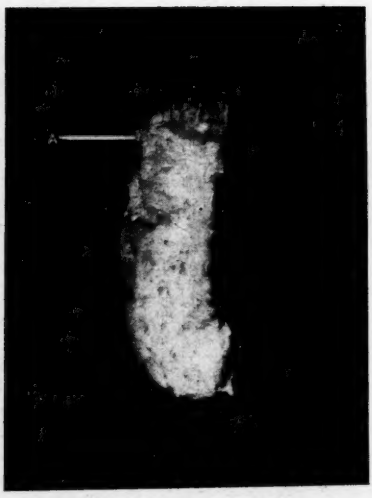

FIG. 2. Two-thirds actual size.

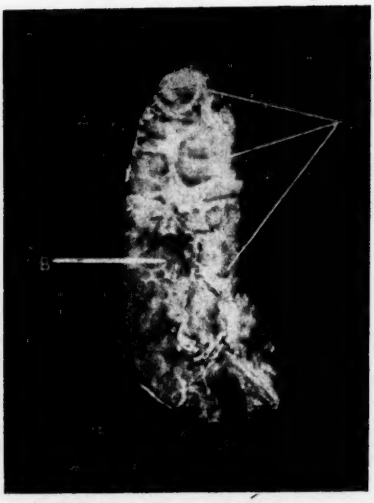

FIG. 3. Three-quarters actual size.

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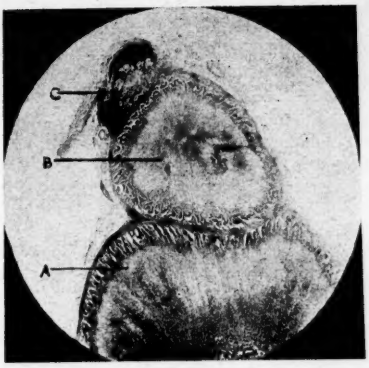

FIG. 4.—x 10.

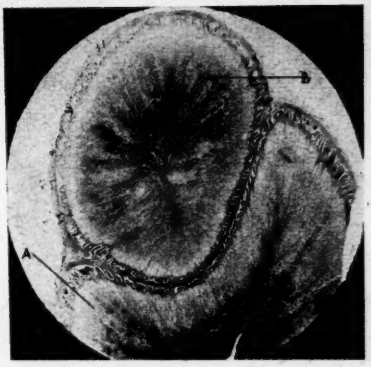

FIG. 7.—x 10.

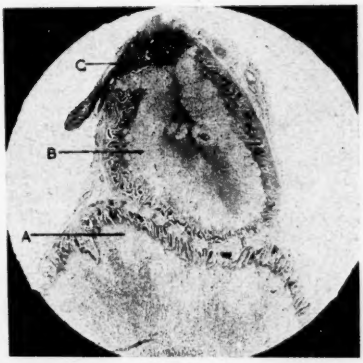

FIG. 5.—x 10.

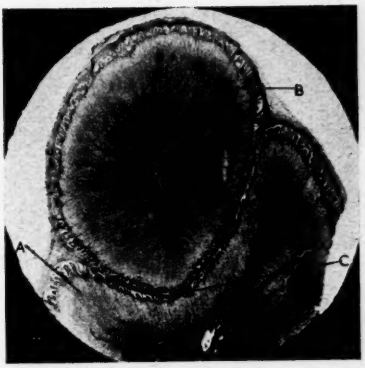

FIG. 8.—x 10.

FIG. 6.—x 10.

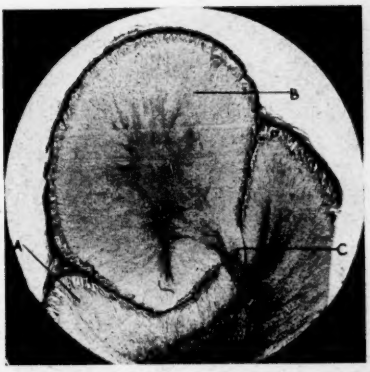

FIG. 9.—x 10.

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FIG. 10.—x 130.

FIG. 11.—x 500.

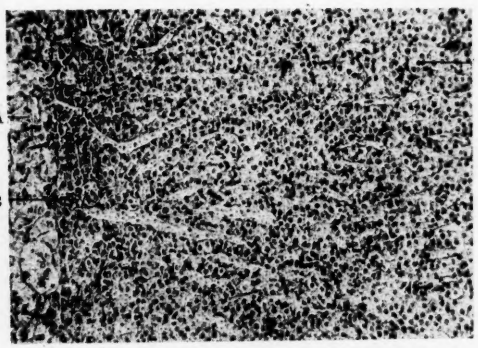

FIG. 12.—x 110.

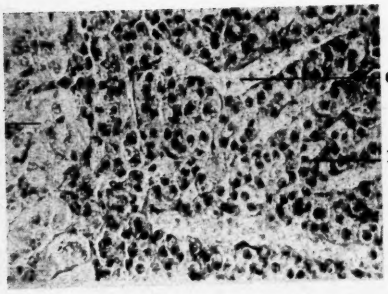

FIG. 13.—x 380.

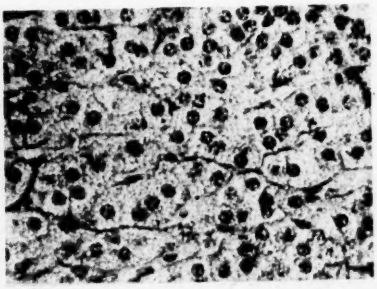

FIG. 14.—x 430.

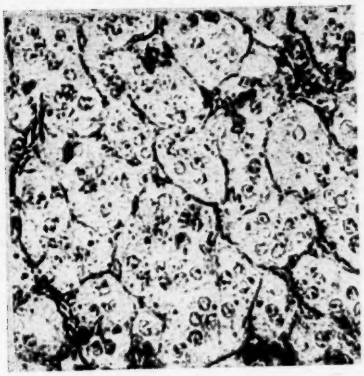

FIG. 15.—x 420.

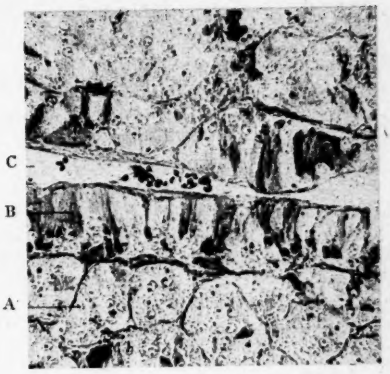

FIG. 16.—x 350.

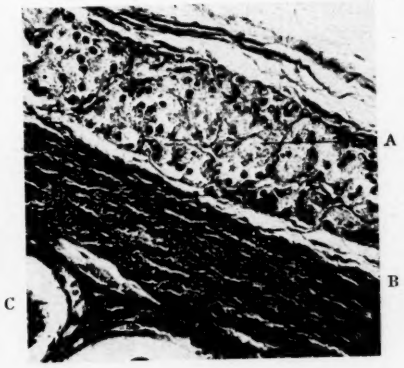

FIG. 17.—x 350.

FIG. 18.—x 340.

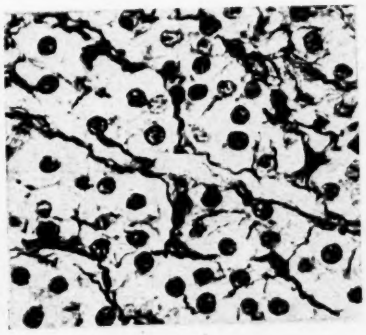

FIG. 19.—x 700.

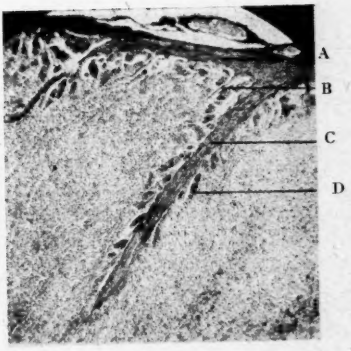

FIG. 20.—x 45.

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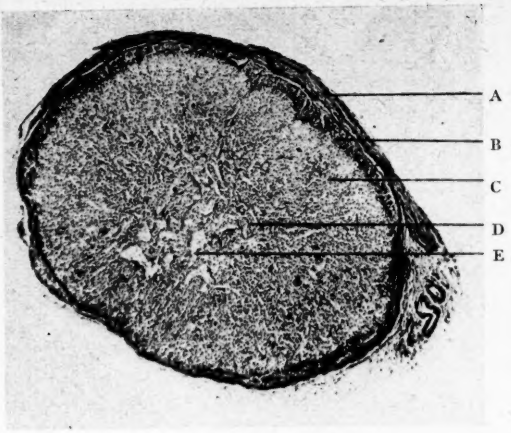

FIG. 21.—x 35.

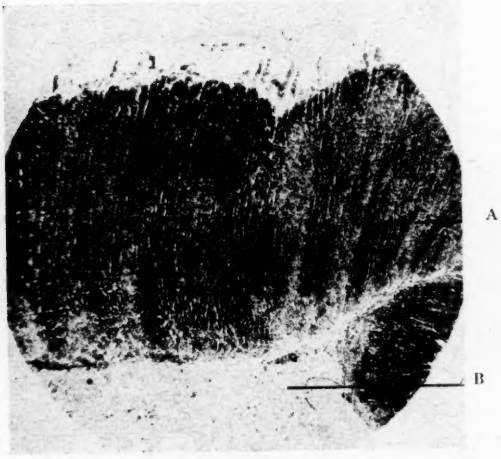

FIG. 22.—x 20.

FIG. 23.—x 270.

clearly defined and is not interdigitated or blended with the cortex as is the case in some animals—gorilla, orangutan, rhinoceros, hippopotamus, giraffe (Grollman). The majority of the cells in the medulla are polyhedral in shape, arranged in bundles distinctly separated from one another by connective tissue and reticular fibres (see Fig. XV). The cells within these bundles have a granular cytoplasm and often contain dark basic-staining inclusions. They show the chromaffin reaction when treated with chrome salts. The nuclei are round and feebly stained with hæmatoxylin. Very often there appears to be a good deal of disintegration of these cells, even when the tissues are rapidly fixed after removal of the gland from the freshly-killed animals, and it is difficult to make out the cell boundaries. The nuclei appear to collect in the centre of the bundles away from the bases of the cells (see Fig. XV). Many large blood sinusoids are present lined only by a single layer of endothelial cells (see Fig. XVI). Immediately outside the endothelial lining there is a layer of columnar cells. (This agrees with Kolmer's findings.) These columnar cells show the chromaffin reaction to a marked degree, some of the cells appearing a much darker brown than the others (see Fig. XVI). Frozen sections stained to demonstrate fat with Sudan IV show there is practically no fat contained within the medullary cells (see Fig. XXII).

A few small nerve cells were found in the medulla (see Fig. XVIII). This statement conflicts with Schaefer's finding that there are no nerve cells within the gland, but, on the other hand, it agrees with the statement by Jordan, who says that in the vicinity of the central vein small nerve trunks are seen and occasional minute ganglia or isolated nerve cells occur along their course (*Textbook of Histology*, Jordan).

Histology of Nodules and Undulations Associated with the Surface of the Gland

As described in the portion of this paper dealing with the macroscopical appearance of the gland, there are at least three types of "nodules" or undulations associated with the surface of the gland:

(1) Large undulations caused by proliferation of the cortical tissue between two of the trabeculæ that divide the cortex into lobes;

(2) Nodules reddish-brown in colour and having a circumscribed appearance;

(3) Small yellow circumscribed nodules;

(4) True accessory adrenal bodies.

Examination of free hand and frozen sections of portions of the glands showing the various types of surface undulations and nodules was first made with a dissecting microscope. As far as could be seen by this method, the undulations on the surface appeared to be caused by proliferation of the cortical tissue between the trabeculæ. The large red-brown nodules consisted of cortical tissue only and were not completely isolated from the main gland, as the macroscopical

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examination might lead one to believe, but connected by a distinct neck of cortical tissue to the cortex of the main gland.

In consequence of the above findings, it was decided to make paraffin blocks of the tissues and prepare serial sections through some of the nodules and undulations on the surface of the glands for a more critical examination.

Serial I. The first series of sections was made through a large red-brown nodule on the anterior extremity of an adrenal from a Clydesdale mare (age 12). The block was cut to show the complete nodule and the cortex and medulla of the main gland. Every fifth section was taken, mounted and stained with hæmatozylin and eosin. The tissue was fixed in Zenker's formol and showed the chromaffin reaction very distinctly. Upon examination of the series it was found that the nodule consisted of cortical tissue only, the three zones of the cortex being clearly shown, surrounded by a connective tissue capsule which completely isolated the nodule from the main gland. There was no sign of any medullary tissue within the nodule. No connection was found between this nodule and the main gland, therefore this nodule was taken to be a true accessory.

Serial II. The next series of sections to be made was from a portion of gland containing another large red-brown nodule taken from the left adrenal of a Clydesdale gelding (age 13). Examination of this series showed the nodule to consist, as before, of cortical tissue only, surrounded by a connective tissue capsule, but it was found that this nodule was directly connected with the main gland by a neck of cortical tissue continuous with the cortex of the main gland (see Figs. VII, VIII, IX).

Serial III. A portion of gland showing a red-brown nodule was taken from the left adrenal of another Clydesdale gelding, 13 years old. On examination, this nodule was found to be covered with a capsule of connective tissue and the glandular substance to consist of cortical tissue showing the three zones, with accessory chromaffin tissue existing as a cap on the summit of the nodule. The sections showed the presence of a distinct neck of cortical tissue connecting the nodule with the cortex of the main gland (see Figs. IV, V, VI).

Serial IV. A block of tissue showing a group of small yellow nodules was taken from the adrenal gland of an 18-year-old Clydesdale mare. Examination of the sections showed that these nodules consisted of cortical tissue only, the smaller nodules showed the Z. glomerulosa only, while the larger showed the Z. glomerulosa and Z. fasciculata lying directly under the capsule. This is probably the reason why these nodules appear yellow in colour, as the Z. fasciculata is characteristically yellow in the fresh unfixed specimen.

Other series. Eight other lots of tissue from adrenal glands showing nodules on the surface were blocked and serial sections cut. In these cases the nodules were found to consist of cortical tissue only and to be connected with the cortical substance of the main gland.

From this the conclusion may be drawn that the nodules and undulations on the surface of the adrenal glands are practically all lobulations of the cortical substance of the main gland, and not true accessory adrenals. Referring to "accessory" bodies which project like warts from the surface of the gland, Grollman says: "This type of accessory is in reality merely a hypertrophic nodule which has formed near the surface of the gland." (Grollman, p. 35.) Weber

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also refers to hyperplastic nodules in human adrenals. Mackay and Mackay refer to compensating hypertrophy of the cortical tissue of one adrenal after experimental removal of the other.

True Accessory Adrenals. These are small bodies completely isolated from the main gland, found embedded in the perirenal fat. Upon microscopical examination they showed a connective tissue capsule with trabeculæ running into the substance of the gland. The glandular substance appeared to consist of cortical tissue only, the Zona glomerulosa poorly developed, the Zona fasciculata and Zona reticularis relatively wide. No medullary tissue was present in any of the true accessory adrenals examined (see Fig. XXI).

Accessory Chromaffin Tissue. During the examination of the serial sections made through the nodules and undulations on the surface of the main glands, it was noticed that in addition to the medulla of the main gland there was present, in some sections, tissue that gave the chromaffin reaction. In most cases this tissue appeared as flat, elongated areas lying in some cases on the surface of the capsule (see Fig. XVII), in others embedded in the capsule of the main gland. This tissue frequently appeared to be associated with the surface undulations and nodules, but it was also seen where no undulations or nodules were present.

In one case (Figs. IV, V, VI) this tissue was seen very closely associated with one of the lobulations of the cortex. In Fig. IV the tissue is seen lying on the surface of the nodule, but separated from it by the connective tissue capsule of the nodule. In Figs. V and VI the tissue is seen in direct contact with the cortical substance of the nodule, the connective tissue capsule now being absent from that area.

The cells of this accessory chromaffin tissue are polyhedral in shape and arranged in more or less spheroidal groups separated from one another by strands of connective tissue. The nuclei of the cells are round, the cytoplasm granular and distinctly brown in colour, due to the chromaffin reaction.

Conclusions

(1) From the limited number of specimens examined, it appears that in young horses, under 10 years, the adrenals are small and their surfaces smooth and uniformly coloured red-brown or yellow-brown.

(2) In horses over 10 years the adrenals are considerably larger and irregularities of the surface appear more frequently.

(3) Histological examination showed that these irregularities of the surface consist entirely of cortex, and are of four distinct types.

(4) True accessory adrenal bodies do not appear to be numerous or large in the horse; such bodies are unconnected with the substance of the main gland.

(5) Accessory chromaffin tissue is present on the surface of the capsule and embedded in the capsule.

Summary

This paper is based on the examination of the adrenals of 18 Clydesdale horses and 4 Cross-Clydesdale horses.

It contains a description of the gross anatomy and histology of the glands.

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An attempt is made to describe the glands in animals considered to be healthy, as a basis for comparison with the adrenals of animals suffering from grass-sickness and certain other conditions.

Particular attention is paid to the histology of the cortex, because of the irregularities frequently noted in the gross anatomy of this structure.

Acknowledgments

Our thanks are due to Col. Sir Arthur Olver, Principal of the College, for his interest and assistance, to Professor Grahame, of the Department of Anatomy, for his supervision and very helpful advice, and to Mr. W. Neill, Technician, Anatomy Department, Royal (Dick) Veterinary College, who prepared the photographs and histological material.

We are also greatly indebted to Mr. Wallis, Penicuik, Midlothian, for his great kindness in providing us with facilities for obtaining the material.

REFERENCES.

1. Carleton, H. M. (1944): "Schaefer's Essentials of Histology."
2. Grollman, A. (1936): "The Adrenals."
3. Jordan, H. E. J. (1930): "A Text-Book of Histology."
4. Kolmer, W. (1918): *Arch. f. mikr. Anat., 91*: 1.
5. MacKay, E. M., and MacKay, L. L. (1926): *J. exptl. Med., 43*: 395.
6. Sisson, S., and Grossman, J. D. (1938): "The Anatomy of the Domestic Animals."
7. Weber, F. P. (1926): *Brit. J. Dermat., 38*: 1.

SOME OBSERVATIONS ON THE INCIDENCE OF BOVINE HELMINTHS IN PLATEAU PROVINCE, NORTHERN NIGERIA

By J. F. A. SPRENT, *Veterinary Research Officer, Nigeria.*

Among the nomadic, native-owned cattle of Northern Nigeria it is commonly observed that certain animals become emaciated and anæmic. Many factors, including protozoal diseases, helminthiasis and under-nutrition, might be responsible for such a condition, and where all such factors probably co-exist it is extremely difficult to investigate the ætiology. One method of investigation is by making a survey of a large number of animals, in which their general bodily condition and their blood picture may be related to the incidence of any one of those factors, thus either eliminating or incriminating that factor, as a possible ætiological agent.

At the Veterinary Laboratory young cattle are slaughtered in the production of anti-rinderpest serum. A number of these animals is purchased every week from the native cattle owners and thus are typical young stock of the nomadic herds which are to be found in Nigeria. Opportunity was taken to make a helminthological survey of a large number of them. On arrival at the Laboratory each animal was weighed, its general condition noted and the blood hæmoglobin estimated. Virulent rinderpest blood was then administered, and a few days later the animal was bled to death. The carcase was searched for helminth parasites and for any lesions which might have been caused by them. About 250 animals were examined in this way at the rate of 20 per month. The purpose of the survey was to detect whether poor bodily condition

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and anæmia were related to the helminth burden. The results of the survey may be conveniently divided into the following headings:

1. Incidence of helminths.
2. Autopsy findings.
3. Hæmoglobin estimations.
4. Discussion.
5. Summary.

1. Incidence of Helminths

At least 23 different species of helminth parasites were obtained from these carcases and were brought back to the United Kingdom for identification. The results are shown in Table 1.

TABLE 1.

Nematodes:
Ascaris vitulorum,
Strongyloides papillosus,
Trichuris globucosa,
Oesophagostomum radiatum,
Bunostomum phlebotomum,
Gaigeria pachyscelis,
Hæmonchus contortus,
Cooperia punctata,
Cooperia pectinata,
Trichostrongylus axii,
Trichostrongylus colubriformis,
Gongylonema verrucosum,
Thelazia rhodesii,
Onchocerca gibsoni,
Setaria cervi.

Trematodes:
Fasciola gigantica,
Dicrocœlium hospes,
Cotylophoron cotylophorum,
Schistosoma bovis.

Cestodes:
Moniezia expansa,
Moniezia benedeni,
Avitellina centripunctata,
Helictometra giardi,
Cysticercus bovis.

Nematodes.—In Table 2 is shown numerical data on the incidence of the five most commonly found intestinal nematodes of cattle. The worms were counted by the dilution method described by Taylor (1938).

TABLE 2.

	Hæmonchus	*Cooperia*	*Trichostrongylus*	*Bunostomum*	*Œsophagostomum*
Percentage of animals infested ...	74	77	25	55	39
Proportion— Males : females...	1 : 1.4	1 : 1.5	1 : 11	1 : 1.8	1 : 1.5

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	Hæmonchus	*Cooperia*	*Trichostrongylus*	*Bunostomum*	*Œsophagostomum*
Average count—					
Males	200	500	less than 100	less than 100	less than 100
Females ...	300	700	300	„ „ 100	100
Maximum infestation—					
Males	2,300	2,400	200	150	400
Females ...	2,100	3,400	1,300	200	500

It will be observed that the nematode burden was usually light. The other nematode species listed in Table 1 and not included in Table 2 were present only sporadically and never in large numbers.

As well as the regular examination of yearling cattle, the fæces of cattle in local herds were often examined for parasitic eggs, as was intestinal material obtained from autopsies in the field. Table 3 shows the earliest and latest ages at which the eggs of certain nematodes were observed in the fæces.

	Earliest age at which eggs appeared in fæces.	*Age at which eggs disappeared from fæces.*
Ascaris	16 days.	5 months.
Strongyloides	13 days.	6 months.
Bunostomum	7 months.	Indefinite.
Trichostrongylid group ...	3 months.	Indefinite.

Trematodes.—Nearly all the livers examined showed at least one specimen of *Fasciola gigantica,* but no heavy infestations were seen. *Dicrocœlium hospes* was usually present, but only in small numbers. Only one animal harboured *Schistosoma bovis,* and about 50 per cent. of the cattle harboured a few specimens of *Cotylophoron cotylophorum.*

Cestodes.—The largest number of tapeworms ever found in one animal was four, although about 15 per cent. harboured at least one.

2. Autopsy Findings

In none of the autopsies performed on native-owned yearling cattle were any lesions found which indicated that helminths were pathologically important in these animals, except for the bite-marks of *Bunostomum phlebotomum* in the upper duodenum, the parasite, however, was never present in large numbers (see Table 2). Young calves, on the other hand, often showed a heavy infestation of *Ascaris vitulorum.* On two occasions the worms caused a blockage of the intestine; in both animals intussusception of the ileum through the ileo-colic aperture with consequent gangrene was observed following treatment with 6 c.cs. turpentine.

3. Hæmoglobin Estimations

The hæmoglobin values ranged from 50 per cent. to 120 per cent. of the Sahli standard. A considerable number of the cattle examined had a hæmoglobin value of less than 70 per cent., and in view of their general symptoms were considered borderline cases of anæmia. It was noticed that the number of cattle below 70 per cent. was greater in the rainy season. This is shown in Table 4, in which is recorded the averaged monthly hæmoglobin percentage of 20 animals per month throughout the year. It will be seen that the values tend to be higher in the dry season than during the rains.

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TABLE 4.

May, 1943	84
June	70

Average hæmoglobin values for each month of the year in nomadic cattle.

Month.		*Average hæmoglobin percentage in 20 animals.*
July		62
August		70
September		78
November ...	Dry Season.	84
December ...		84
January, 1944 ...		93
February ...		97
March		101
April		88

The average number of the nematodes mentioned in Table 2 present in the intestines of each animal with a hæmoglobin value below 70 per cent. was 1,360, while the average number per animal carried by those whose hæmoglobin value was above 70 per cent. was 1,350. A further comparison of the total number of individual species of nematodes per animal with the hæmoglobin values also showed no correlation; the figures are not quoted.

4. Discussion

The question to be decided is whether the anæmia commonly observed in nomadic yearlings in Northern Nigeria can be attributed to helminthiasis: from the epidemiological viewpoint this would seem very unlikely, owing to the very extensive grazing range of these cattle, compared with the limited range on an English farm, and the author's findings tend to bear out such an inference. The average helminth burden of each animal was light and no lesions attributable to helminths were observed in the carcases. The hæmoglobin concentration in the blood seemed to bear no relation to the total nematode burden nor to the numbers of individual species of nematode harboured by the animal.

Of the three ætiological factors originally suggested, protozoal diseases and under-nutrition remain to be considered. It is well known in Nigeria that during the dry season the cattle get into very poor bodily condition and improve strikingly on the advent of the rains, and this is due, doubtless, to the deterioration of the pastures which results from the long dry period. The observations on blood hæmoglobin described above show, however, that the incidence of anæmia is higher in the wet season, when the pastures are at their best. Thus, while the emaciation observed during the dry season is no doubt due to under-nutrition, this factor can play little part in the genesis of the anæmia so commonly observed in young stock.

A comparison may be made between the nomadic cattle and the cattle at the Veterinary Stock Farm, where the same breed of animal has been placed in permanent limited pastureland. In the same way, many of the native cattle-owners are begining to settle down in one locality and limit the grazing range of their cattle. It is interesting, and perhaps illuminating, to note that in Stock Farm cattle of the

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same age as the nomadic animals surveyed in this paper, the hæmoglobin values are lowest in the dry season, and the average number of *Hæmonchus contortus* and *Bunostomum phlebotomum* found in their carcases was about five times greater than the average among the nomadic cattle. Here the anæmia is probably associated with the helminth burden (Sprent, in press). This finding points to the danger which lies ahead when the native herdsmen begin to settle on a large scale and employ permanent pastures for their cattle. If the grazing range is cut down and pastures are over-grazed certain species of helminth parasite may become very prevalent, and thus the poor nutrition of the cattle in the dry season, together with heavy helminth infestation, will result in considerable losses.

This comparison is also of possible value in elucidating the cause of the anæmia of nomadic cattle. While the Stock Farm cattle are regularly dipped, the nomadic cattle are not, and nearly all those examined carried ticks. The number of ticks was higher during the rains than in the dry season, as is usually found, and consequently infections with Anaplasma and Babesia must be most prevalent in the rainy season. Although blood smears were made from all the cattle examined and these parasites were never found, it does not preclude the possibility that by constant reinfection a premunity was established, and that the parasites were present in some body tissue and exerted a chronic effect on the blood. This would explain why anæmia was observed in nomadic cattle in the rainy season and not in the dry season, and why anæmia was not observed in Stock Farm cattle in the rainy season.

Although the Tsetse fly does not occur on the Plateau, where these observations were made, trypanosomiasis may have played some part in these findings. At the beginning of the rains the herdsmen bring their cattle up on to the Plateau from places such as the Benue Valley, where the fly is present, and some of the animals examined during the rains may have been affected with trypanosomiasis. Two and a half per cent. of the cattle showed *Trypanosoma vivax* in the blood during the rains. Anæmia associated with trypanosome infection would thus show itself during the rains in this particular localty.

5. Summary

A survey of helminths of Zebu cattle examined at the Veterinary Laboratory, Northern Nigeria, is described. A list is given of the species found and the incidence of certain of the intestinal nematodes is recorded.

Autopsy examination of 250 cattle failed to show any important lesions caused by helminths, except cases of intussusception in two young calves associated with large numbers of *Ascaris vitulorum* and the bite marks of *Bunostomum phlebotomum*; the latter were, however, only present in small numbers.

Examination of the hæmoglobin values of the blood of these cattle showed that the values are lower in the rainy season (May to September) than during the dry season. The anæmia was shown to bear no relationship to worm infestation. The suggestion is put that it is due to chronic reinfestation by tick-borne protozoa. It is pointed out, however, that when the grazing range of these cattle is cut down helminthiasis is likely to assume an important rôle.

REFERENCES.

Taylor, E. L. (1938): *Veterinary Record, 50*: 1329.
Sprent, J. F. A.: In press.

CAPON PRODUCTION BY THE USE OF SYNTHETIC ŒSTROGEN (STILBOESTROL)

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Introduction.

THE practice of caponising cockerels destined for the table is undoubtedly very ancient.

Burton (1859) refers to caponising of cattle and poultry as having prevailed in pre-Arabic times. The practice of caponising poultry was referred to in Britain by Aelfric, a Winchester monk, whose Anglo-Saxon writings (c. 1000 A.D.) were among the finest of his time. It was also mentioned by Langland in his "Piers Plowman" (1362), by William Caxton, first English printer, in his translation from the Dutch of Gerrard Leeu's "Reynard the Fox" (Gonda, 1479), and in Shakespeare's "As You Like It" (1600). In Elizabethan times, capons were esteemed not only for eating but for the preparation of capon-beer, which was beer in which capon flesh had been cooked.

In fact, the general impression given by old English writings is that capons were eaten to almost as great an extent as were non-castrated chickens, though perhaps their culinary excellence when compared with ordinary cockerels led to this emphasis.

Operative mortality in caponising, even in very skilled hands, is far from negligible, and the setback caused to the birds is an additional financial liability in their production, the consequence being that capons have been for some considerable time past a luxury article.

The principal advantages of caponising are:—

(1) Final dressed weight of bird is very great.

(2) The temperament of the neutered birds is such that their activity is lessened, and greater live-weight gains per lb. of food may be expected. At the same time difficulties and losses due to fighting, etc., are eliminated.

(3) The finish of the birds is excellent; fat deposition (intramuscular as well as mass deposits) is very much increased, and the overall quality of the dressed carcase is superior to anything obtainable by other means.

With the advent of cheap synthetic œstrogens it was considered worth while to see whether the partial "neuterising" which can be obtained by their means would enable capons or near-capons to be produced without the necessity for operative procedure.

Since the work reported here was commenced, the results of a considerable number of trials carried out in U.S.A. have been published. Lorenz (1943, 1945) has used stilboestrol principally as implanted pellets. He found that oral administration was very much less efficient than implantation in terms of stilboestrol dosage, and his findings have been confirmed by Jaap and Thayer (1944), and Thayer, Jaap and Penquite (1944). The latter workers found diethylstilboestrol dimethyl ether very much more potent orally than stilboestrol. The effects of administration of the hormone were not exactly and entirely those seen in capons, because whereas in capons the effect tends to be due to absence of masculinising hormones, in the œstrogen-treated birds production of androgens still continues on a reduced scale, but in the presence of appreciable amounts of feminising hormone. The final results, however, from a marketing point of view, were similar

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in increased rate of gain of weight, increased final weight, and better finish in respect of increased depot and intramuscular fat deposition. No studies were made, apparently, of the weight gain per unit weight of food.

Experimental

(1) *Œstrogen used and its incorporation in the feed.*

Stilboestrol was used throughout the experiment. It was obtained in finely powdered form and a weighed quantity was still further comminuted in a mortar with three or four times its weight of powdered calcined ferric oxide. The purpose of the ferric oxide was to act as an inert marker in the subsequent mixing and to characterise the final master mix by its intense colour.

Sufficient wheat flour was then added to the stilboestrol-ferric oxide mix to bring the concentration of stilboestrol to 1 in 1,000 (1 mg./g.), and this was mixed thoroughly in an end-over-end mixer for 15 minutes, by which time incorporation was obviously very even and thorough.

The stilboestrol master mix was added to the mash (used throughout the experiments) at the rate of 1 oz. per 7 lb. of mash, representing 1 mg. of stilboestrol per 4 oz. of mash.

The composition of the mash was as follows:—

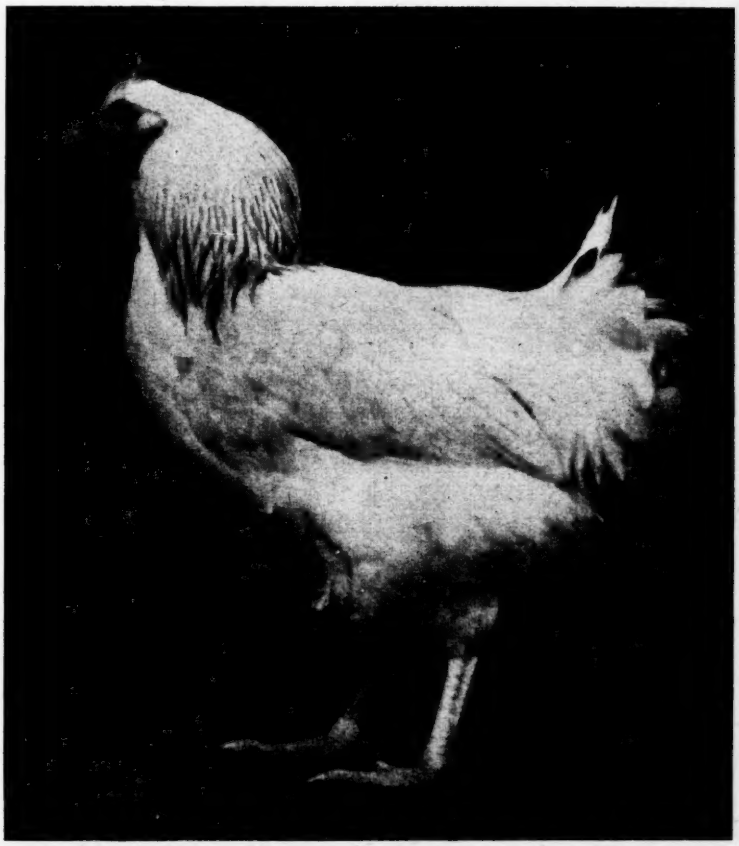
Wheatings	36 per cent.
Ground Oats	15 „ „
Rice Meal	15 „ „
Bran	12 „ „
Fish Meal	10 „ „
Dried Skim Milk	5 „ „
Dried Yeast	2 „ „
Lucerne Meal	5 „ „
	100 „ „

With the object of reducing the expense of œstrogen feeding it was decided in the first instance to use the stilboestrol in the later stages of growth to endeavour to give a "capon finish" to the birds. Therefore cockerels of 17 weeks of age were used, divided at random into two groups of 15 and 16 birds respectively, pen I having the stilboestrol addition, pen II serving as controls. A small quantity of corn was fed in addition to the mash, the latter being fed principally wet (moistened with skimmed milk).

The food consumed weekly per bird during the 7 weeks experimental period is shown in Table I.

TABLE I. *Food Consumption.*

	Pen I (Stilboestrol).			Pen II (Control).		
	Wet Mash.	*Dry Mash.*	*Corn.*	*Wet Mash.*	*Dry Mash.*	*Corn.*
Week 1 ...	28 oz.	—	7 oz.	28 oz.	—	7 oz.
„ 2 ...	28 oz.	4 oz.	7 oz.	28 oz.	4.5 oz.	7 oz.
„ 3 ...	28 oz.	6.66 oz.	7 oz.	28 oz.	1.75 oz.	7 oz.
„ 4 ...	28 oz.	11.46 oz.	7 oz.	28 oz.	8.25 oz.	7 oz.
„ 5 ...	42 oz.	5.86 oz.	7 oz.	42 oz.	5.25 oz.	7 oz.
„ 6 ...	42 oz.	3.06 oz.	7 oz.	42 oz.	4.63 oz.	7 oz.
„ 7 ...	42 oz.	1.6 oz.	7 oz.	42 oz.	3.5 oz.	7 oz.
	Grand total ... 270 oz.			Grand total ... 266 oz.		



Typical bird from Pen I (Stilbœstrol).

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Typical bird from Pen II (Control).

The results of this feeding are shown in Table II. No untoward effects of a toxic nature were observed in the stilboestrol fed group.

TABLE II. *Weights of individual birds in the experiments.*

Pen I (Stilboestrol).						Pen II (Control).					
Bird No.	*Initial Wt.*		*Final Wt.*		*Gain.*	*Bird No.*	*Initial Wt.*		*Final Wt.*		*Gain.*	
	lb.	*oz.*	*lb.*	*oz.*	*lb.* *oz.*		*lb.*	*oz.*	*lb.*	*oz.*	*lb.*	*oz.*
464	5	10	6	13	1 3	463	5	12	6	11		15
469	5	5	6	8	1 3	467	5	10	6	8		14
472	5	9	6	13	1 4	470	6	3	7	8	1	5
474	6	14	7	8	10	473	5	12	6	8		12
476	5	11	6	9	14	475	5	12	6	9		13
478	5	11	6	14	1 3	477	5	2	6	0		14
481	5	10	6	12	1 2	479	5	10	6	9		15
484	5	2	6	5	1 3	480	5	2	6	6	1	4
486	6	0	7	0	1 0	482	6	6	7	14	1	8
487	5	9	6	13	1 4	485	5	7	6	15	1	8
490	6	5	8	2	1 13	488	5	10	6	8		14
494	6	3	7	8	1 5	492	5	12	6	13	1	1
495	5	15	7	9	1 10	493	7	5	7	13		8
497	5	10	7	1	1 7	496	5	5	6	12	1	7
498	5	8	6	14	1 6	499	6	2	6	14		12
						500	5	13	6	8		11
Total Initial Wt. ...			86 lb. 10 oz.				92 lb. 11 oz.					
„ Final Wt. ...			105 lb. 1 oz.				108 lb. 12 oz.					
„ Gain in Wt. ...			18 lb. 7 oz.				16 lb. 1 oz.					
Mean Individual Gain ...			1 lb. 3.66 oz.				1 lb. .06 oz.					

The significance of the difference between the mean individual weight increases of the two pens of birds was estimated by the "t" test, using the formula:

$$t = \frac{x_1 - x_2}{s\sqrt{\frac{1}{N_1} + \frac{1}{N_2}}}$$

Where x_1 and x_2 were the mean weight gains in the two pens (in oz.), N_1 and N_2 were the numbers of birds in the respective pens, and s was obtained by summing the squares of the deviations from the two means, dividing by $N_1 + N_2 - 2$ (the degrees of freedom) and finding the square root.

s in this instance = 4.7434, and thus $t = \frac{3.598}{4.7434 \times \sqrt{1/15 + 1/16}} = 2.11$

From Fisher's (1936) "T" tables, the value of t on the .05 level of significance (i.e., 95 times out of 100) for 29 degrees of freedom = 2.045, and therefore the difference between the mean weight increases of the birds in the two groups is significant. During the dressing of the birds the testicles were weighed, those of the birds in Pen I being less than half the size of those in the control group. It was also very obvious that carcase quality had been improved in the manner

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described by Lorenz (1945), the birds possessing in large measure the desirable qualities of capons (see Plate I).

Discussion

From the American work already referred to and the results of this experiment it is abundantly clear that caponising of cockerels by administration of synthetic œstrogens is an accomplished fact. It is likewise obvious that the method of administration of the hormone should be via the mash, because (1) it is essential that any possibility of a hormone residuum of any size should be avoided, and therefore implantation or injection of œstrogen is ruled out, (2) labour costs of individual dosage and attention to birds would negative commercial advantages otherwise obtainable by the use of œstrogen.

In the present experiment it is clear that optimal effect was not attained—the halving of testicle size only, indicated that higher dosage of œstrogen (or the use of an œstrogen of greater potency for the fowl) is necessary to achieve the equivalent of full caponising. Nevertheless, the effect obtained was sufficient to prove the feasibility of the method, and further showed that an increased economy in the conversion of foodstuff into liveweight gain results incidentally from the use of œstrogen. This point has so far not been observed by the American workers, though Thayer, Jaap and Penquite (1944) must have had the probability of a lowered metabolic rate in mind when they fed thiouracil in addition to œstrogen in certain experiments.

It remains to be shown which synthetic œstrogen is most effective for poultry, and which is most desirable on grounds of economy and of stability in the mash; also whether it is desirable and economically sound to feed œstrogen from, say, 5 weeks to killing, or only during the last few weeks of fattening, to provide a better finish to the birds than would otherwise be obtained.

Summary

(1) The feeding of stilboestrol at the rate of 1 mg. rising to 1.5 mg. per bird per day to 17-week cockerels rapidly induces changes in them simulating in every respect the effects of caponisation.

(2) An improvement was observed in the utilisation of food by the œstrogen fed birds.

(3) Optimal results were not obtained, and the possibility of the use of other œstrogens "per os" is discussed.

Acknowledgment

Our thanks are due to Mr. C. E. Elms for photographing the birds.

REFERENCES

1. Burton, R. (1859): Roy. Geog. Soc., *29*, 350.
2. Fisher, R. A. (1936): *Statistical Methods for Research Workers*, 6th Edition.
3. Jaap, R. G., and Thayer, R. H. (1944): "Oral Administration of Œstrogens in Poultry." *Poultry Sci., 23*, 249-251.
4. Lorenz, F. W. (1943): "Fattening Cockerels by Stilbœstrol Administration." *Poultry Sci., 22*, 190-191.
5. Lorenz, F. S. (1945): "The Influence of Diethylstilbœstrol on Meat Deposition and Fat Quality in Chickens." *Poultry Sci., 24*, 128-134.
6. Thayer, R. H., Jaap, R. G., and Penquite, R. (1944): "Fattening Chickens by Feeding Œstrogens." *Poultry Sci., 23*, 555.
7. Tippett, L. H. C. (1941): *The Methods of Statistics*, 3rd Edition, pp. 114-115.

Abstracts

SEX HORMONES IN VETERINARY PRACTICE*

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It is the purpose of this paper to discuss the physiology of the sex hormones and to correlate this with the products now available to the veterinarian. Some of the difficulties encountered in making such an analysis from the existing literature must be pointed out. The most outstanding of these difficulties are the large amount of contradictory information that is found on this subject and the relative paucity of controlled experiments with these various products on the domestic species. We should be cautious in accepting the result of any experiment as evidence until it has been widely supported. Too often we are led astray by assuming that a change following the administration of a drug has resulted from the action of the drug. This can be assumed only when the action is the same in repeated trials, or when appropriate controls are used. Confusion has also resulted from the fact that much of the knowledge concerning sex hormones has been obtained from experiments carried out on small laboratory animals. Such information does not necessarily apply to the larger species, although it is in many instances a valuable background from which to experiment. Dosage and the influence of the route of administration of sex hormones have not been investigated thoroughly. Some of the products have different actions depending on whether they are given subcutaneously, intramuscularly, intravenously, or per os. Further, the sex hormone physiology of domestic animals is certainly not thoroughly understood. There are no adequate clinical methods for determining the levels of the hormones in the body, so that a deficiency is often difficult to detect and frequently other etiological factors may enter into what appears to be simply a hormone deficiency. All of these factors have contributed to the failure and confusion that sometimes accompany the therapeutic use of these products. However, it is our duty not to despair, but rather to use these agents as widely as practicable. There is no doubt that hormone therapy can be an important and indispensable aid to the practising veterinarian. It is up to him to exploit the possibilities of these agents, reporting both successes and failures, so that ultimately order can be made and practical knowledge gained. It should be pointed out that one must be careful about his source of information concerning drugs. The various drug companies have made laudable efforts and valuable contributions in veterinary therapeutics. This we should recognise and appreciate. At the same time we should realise that some firms will base claims on insufficient evidence. Since such information is very widely distributed through advertising, it is important for one to weigh the evidence behind such claims very carefully.

Chart I (slightly modified from the original given by the author) summarises some of the important facts regarding the physiology of the sex hormones.

CHART I

Hormone	Chief Source in the Animal Body	Organ acted on by Hormone	Interrelations	Function
Natural Œstrogen	Follicle (Placenta)	Tubal mucosa Mammæ (ducts)	Progesterone Anterior Pituitary	1. Induces œstrus 2. Secondary sex characters 3. Growth of mammæ (ducts)

* *University of Pennsylvania Bulletin* Extension Quarterly No. 98, April, 1945.

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Hormone	Chief Source in the Animal Body	Organ acted on by Hormone	Interrelations	Function
Progesterone	Corpus luteum (Placenta)	Uterus Mammæ (alveoli)	Œstrogen Anterior Pituitary	1. Essential to gestation 2. Progestational changes 3. Growth of mammæ (alveoli) 4. Suppression of œstrus, ovulation, and uterine motility
Gonadotropic F.S.H. (Follicle Stimulating (Hormone)	Anterior Pituitary	Ovary Testes	Œstrogen	1. Initiates sexual activity 2. Stimulates follicle—maturation and œstrin. 3. Causes ovulation 4. Stimulates epithelium of seminiferous tubules —spermatozoa
L.H. (Luteinising Hormone)	Anterior Pituitary	Ovary Testes	Œstrogen	1. Luteinisation—progestin 2. Inhibits œstrus and estrin 3. Stimulation of interstitial cells of testes—testosterone
Prolactin	Anterior Pituitary	Mammæ	Œstrogen Progesterone	1. Induces milk secretion
Mammogens	Anterior Pituitary	Mammæ		1. Induce mammary growth
Duct Alveolar		ducts alveoli		
Testosterone	Testes (Leydig cells)	Sperm	Anterior Pituitary	1. Secondary sex characters 2. Prevents atrophy of accessory sex organs 3. Affects motility of the sperm 4. Libido

The Regulation of the Sexual Cycle.—The manner in which the rhythm of the sexual cycle is maintained is not completely understood. Various theories have been advanced from time to time. Most of these have one thing in common, i.e., that the anterior lobe of the pituitary gland is the directing influence. Just how this is brought about is a matter for speculation. Among the facts that point to the anterior pituitary as the centre of control are these two observations. 1. A rhythmic change in the staining reaction of the cells of the gland can be demonstrated that is associated with the sexual cycle. There are acidophilic and basophilic cells found in the anterior pituitary. Secretory activity of the basophils is associated with the follicular phase of the cycle; and secretory activity of the acidophils is associated with the lutein phase. 2. Cyclic changes in the amount of active substance in the anterior pituitary gland have been observed.

The above concept plus the known facts concerning the interrelationship of the ovaries and the anterior pituitary have led to the theory that it is just this interdependence that controls the sexual cycle. Thus the regulation of the œstrous cycle can be explained in this manner. The F.S.H. of the anterior pituitary stimulates the ovary to produce œstrogen. The resulting high level of œstrogen depresses the F.S.H. production of the anterior pituitary and in this way limits its own production. The corpus luteum that is developing at this time further inhibits the action of the F.S.H. that remains. The corpus luteum development is initiated by the L.H. of the anterior pituitary, the production of which may be secondary to the depression of the F.S.H. production. These changes result in a drop in the œstrogen level of the blood, thus removing the inhibitory influence from production of the F.S.H. At this time the corpus luteum begins to degenerate and this further removes an inhibiting influence on

the F.S.H. production. Then the cycle starts over again with stimulation of the ovary to produce œstrogen.

Obviously, the above explanation is sketchy and is based on assumptions that are not necessarily true. More information concerning the interrelationships between the various sex glands must be had before an accurate explanation can be made.

Some Miscellaneous Facts of Interest.—A relationship between the oxytocic principle of the posterior pituitary (pitocin) and the ovarian hormones has been found. Œstrogen potentiates the action of pitocin on the uterus, and progestin depresses the response of the uterus to pitocin.

It is well known that both male and female sex hormones (œstrin and testosterone) are produced in each sex. For example the urine and testes of adult males contain œstrogen and the urine of the stallion contains more œstrogen than that of the pregnant mare. That androgen is produced in the ovary is indicated by the fact that when an ovary is grafted to the ear of a castrated male rat the prostate is maintained in a normal state rather than undergoing the changes typical of castration. The relative levels of these substances are sometimes expressed as the androgen/œstrogen ratio. This ratio is two to five times higher in the male than in the female.

Certain œstrogenic substances are carcinogenic. For instance, some of the œstrogenic benzanthracene compounds when painted on the skin will produce cancerous growth. It has been pointed out that the cellular growth which occurs during œstrus resembles early malignant growth. This relationship is further demonstrated by the behaviour of mammary tumours in the bitch. It is stated that mammary tumours are not as common in spayed as in normal females. Increased growth of these tumours has been noted at the time of œstrus and it is thought that this is due to the increased œstrogen level at this time. Further, ovariectomy in mice of a strain with a high incidence of mammary tumours greatly reduced the incidence of the disease. The observation has been made clinically that the administration of male sex hormones to bitches with mammary tumours will reduce the size of these tumours and, it has been claimed, sometimes they will be removed entirely. These observations are in line with the fact that the male and female sex hormones exert an antagonistic action to one another (probably indirectly by depressing the gonadotropic activity of the anterior pituitary). It should be pointed out that while œstrogenic substances are carcinogenic in large doses, their use in therapeutic doses does no harm.

Sex Hormone Products Available to the Veterinarian.—Although there are many sex hormone products available, only those most widely used and for which doses have been determined will be considered. These products have been classified according to their actions and their sources as follows:—

Gonadrotropic Hormones	*Œstrogenic Hormones*	*Androgenic Hormones*
1. Anterior Pituitary Extract	1. Natural Œstrogens	1. Natural Androgens
2. Equine Gonadotropin	2. Synthetic Œstrogens	2. Synthetic Androgens
3. Chorionic Gonadotropin		

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Gonadotropic hormones: those substances obtained from the anterior pituitary gland and elsewhere, that have a stimulant effect upon the gonads.

Œstrogenic hormones: those substances obtained from the ovaries and elsewhere, that cause œstrus-like growth in the tubal mucosa.

Androgenic hormones: those substances obtained from the male gonads, that have the action of the male sex hormone.

The Commercial Sources of Sex Hormones

The Gonadotropic Hormones

1. Anterior Pituitary Extract—obtained by extraction from the fresh anterior pituitary gland of cattle, sheep, and horses.
2. Equine Gonadotropin—obtained from the serum of pregnant mares.
3. Chorionic Gonadotropin—obtained by extraction from the urine and placenta of pregnant women.

The Œstrogenic Hormones

1. Natural Œstrogens—extracted from the urine and placenta of pregnant women, and urine of pregnant mares.
2. Synthetic Œstrogens (e.g., diethylstilbœstrol)—produced by chemical synthesis.

The Androgenic Hormones

1. Natural Androgens—crystalline testosterone extracted from bull testes.
2. Synthetic Androgens—crystalline testosterone synthesised from the steroids.

CHART II

Preparation	Source	Chief Actions	Some Suggested Uses
Anterior Pituitary Extract (anterior pituitary solution)	Extract of fresh gland	Follicle stimulation & luteinisation	1. Sexual inactivity in otherwise normal males and females 2. Anœstrum 3. Cryptorchidism 4. Prolongation of lactation 5. Acetonemia
Equine Gonadotropin (pregnant mare serum)	Serum of pregnant mare	Follicle stimulation	1. Sexual inactivity in otherwise normal males and females 2. Persistent corpus luteum 3. Anœstrum
Chorionic Gonadotropin	Human pregnancy urine	Luteinisation. Hastens descent of testes	1. Nymphomania 2. Ovarian cysts 3. Cryptorchidism
Estrone (Theelin)	Pregnancy urine	Produce heat	1. Anœstrum
Estriol (Theelol)		Estrus growth	2. Pyometra
Estradiol	Follicular fluid	in tubal M.M.	3. Mummified fetus
		Influence ant.	4. Retained placenta
Amniotin	Pregnant mare urine	pit. activity	5. Vaginitis
Diethylstilbestrol	Synthetic	Inc. uterine motility	6. Stimulate lactation in dry or virgin animals 7. Urinary incontinence
Testosterone	Testicular tissue		1. Impotence 2. Cryptorchidism
Posterior Pituitary Injection	Aqueous extract of posterior pituitary	Stim. uterine motility Vasoconstriction Anti-Diuretic action	1. Stimulate uterine motility 2. Retained placenta

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Prolactin, the lactogenic hormone of the anterior pituitary, is available commercially as a water soluble powder extracted from bovine anterior pituitary glands. It has been used experimentally to prolong the milk flow in the declining phase of lactation.

Chart III briefly summarises the chief uses of *Anterior Pituitary Extract* thus far found for this product.

CHART III

USE OF ANTERIOR PITUITARY EXTRACT

Condition	Administration and Dosage	Results
Anœstrum	Cow and Mare 5-10 cc* I.M.**	Œstrus in 3-4 days. 2nd injection in 20 days if necessary
	Ewe and Sow 2-4 cc* I.M.	Ovulation. A 2nd injection in 16-17 days may be needed to produce œstrus.
	Dog and cat ½-1 cc* I.M.	May produce œstrus
Cryptorchidism	Injections every other day I.M.	Less effective than chorionic gonadotropin
Acetonemia	Cow 10 cc* I.M.	Appears to be helpful in some cases
Prolong lactation	Cow 10 cc* repeated I.M.	Prolongs milk flow in later stages of lactation. Contraindicated in pregnant animals

*Containing 1.25 grams of fresh gland per cc.
**I.M.—Intramuscular.

Anœstrum.—The use of anterior pituitary extracts in the treatment of anœstrum has been done largely under experimental conditions. Successful results have been reported with anœstrum due to ovarian hypofunction. Apparently, the extracts act by virtue of their follicle stimulating properties in this condition. However, to make the statement that anterior pituitary extracts in general are chiefly follicle stimulating in nature is not correct, although earlier reports in the literature point to that. Whether anterior pituitary extract has chiefly a follicle stimulating effect or chiefly a luteinising effect on the ovary depends on three factors: 1, the source of the product; 2, the method of extraction; 3, the method of administration.

Thus it has been stated by Parkes that extracts of bovine origin are largely luteinising; those from sheep and swine chiefly luteinising; and those from horses chiefly follicle stimulating in effect. Casida, *et al.* have shown that unfractionated extracts from sheep pituitaries when administered to the cow have chiefly a follicle stimulating action subcutaneously and chiefly a luteinising effect when given intravenously. Under experimental conditions it has been possible to so fractionate anterior pituitary extracts that their action is chiefly either follicle stimulating or luteinising. Such fractionated products are to be expected in the future since there is active investigation being carried on in that direction at present. Indeed, there are some products now for which the manufacturers claim a follicle stimulating or a luteinising action as the principal effect.

Cryptorchidism.—The successful use of anterior pituitary extracts in cryptorchidism in humans (boys) has been reported. However, it seems that gonadotropin of chorionic origin is more efficacious in this respect than anterior pituitary extracts.

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Acetonemia.—The use of anterior pituitary in acetonemia, based on the relationship of the anterior pituitary to carbohydrate metabolism, appears to have been helpful in some cases but actually has little to support it.

Prolonged Lactation.—This action of anterior pituitary extract has been reported to be effective in the later stages of lactation. The effect is apparently due to the prolactin contained in the extract. Probably the other factors in the extract contribute to this action, also.

The uses of *Equine Gonadotropin* are briefly summarised in Chart IV.

CHART IV

USE OF EQUINE GONADOTROPIN

Condition	Administration** and Dosage*	Results
Anœstrum	Cow and mare 1000 RU (20-30 cc) Ewe 250 RU (5 cc) Doe 250 RU (5 cc) Sow 500 RU (10 cc) Bitch 250-500 RU (5-10 cc) SC	Œstrus in 3-4 days. Ovulation may occur without œstrus. A 2nd injection inc. 50% over 1st. Should be given at an interval equal to length of œstrus cycle.
Impotent male	Bull 1500 RU Stallion (30 cc) SC Ram 500 RU Buck (10 cc) SC Boar 750 RU (15 cc) SC Dog 375-750 RU (7½-15 cc) SC	Increase sexual desire.

*Doses given in cc.—Gonadin.
**Some mild cases of anaphylxis have been reported in cattle.
SC—Subcutaneous.
RU—Rat units.

The earlier work with this product was done on sheep and the chief action seemed to be follicle stimulating. However, it has also been reported to have mainly a luteinising effect under certain conditions. Here again it is difficult to definitely state in which conditions this product should be used. Considering the information that has thus far been presented it would seem best to class pregnant mare's serum as predominantly follicle stimulating in action, and to recommend its use in those conditions in which such an action is indicated. Its value in producing œstrus in anœstrum due to hypofunctioning ovaries is fairly well established. It has been used in impotence in the male because of the stimulating effect of its activity on the testes.

Chart V summarises the chief uses thus far established for Chorionic Gonadotropin in veterinary medicine.

CHART V.

USE OF CHORIONIC GONADOTROPIN.

Condition	Administration and Dosage*	Results
Nymphomania	Cow 10,000 IU I.M.	Become quiet in 24-48 hours
Cystic ovaries	Cow 10,000 IU I.M.	Ovaries normal in 3-5 days
Cryptorchidism	Horse 250-500 IU every other day for 7-10 days. Dog 250 IU twice weekly for 6-8 wks. I.M.	Enlargement and descent of testes

*IU—International units; I.M—Intramuscular.

Its effect on the ovary is chiefly that of luteinisation. This explains its action in the treatment of nymphomania due to cystic ovaries. In this condition the follicles apparently are functioning supernormally and converting them to corpora lutea removes the cause of the condition. Cystic ovaries not associated with nymphomania have been described as a condition in which there is a cyst of the corpus luteum. Favourable results have been reported in this condition also, with the use of chorionic gonadotropin.

This product has been used in treating cryptorchidism and seems to be the most effective agent, according to results in humans. This action is indicated by the fact that the preparation when injected into immature monkeys will cause the premature descent of the testicles. It is pointed out that chorionic gonadotropin will be ineffective in those cases in which there is some mechanical interference with the descent of the testes. However, since it increases the size of the parts, thus favouring subsequent surgery, it is a useful treatment in this condition whether or not actual descent is obtained.

Chart VI outlines the chief uses of diethylstilbœstrol.

CHART VI
USE OF DIETHYLSTILBŒSTROL

Condition	Administration and Dosage*	Results
Retained placenta	15-20 mgm. I.M.	Increases circulation, secretion, epithelial growth & motility of the uterus. Loosens placenta & cleanses uterus.
Anestrus	Heifer 10 mgm. I.M. Cow & mare 20 mgm. I.M. Sow 5 mgm. I.M. Ewe 3 mgm. I.M. Bitch 1 mgm. I.M.	Usually produces œstrus in 2-3 days.
Mummified fetus	Cow 25 mgm. I.M.	Œstrus in 24 hours. Expulsion of fetus & membranes in 3-6 days.
Pyometra	Cow 25 mgm. I.M. if C.L. is present; 10.15 mgm. if C.L. not present.	Evacuation of pus in 24 hours. Further evacuations during next few days.
Lactation	Cow 5-20 mgm. I.M. twice weekly for 5-8 weeks.	Udder & teat growth & premature lactation.
Urinary incontinence	Spayed bitch 1 mgm. for 3 days followed by 1 mgm. every 3rd day P.O.	Relief of incontinence.

*IM—Intramuscular; PO—Peros.

This synthetic hormone produces the same physiological effects as the natural œstrogens without any marked toxic action. Since it is not as readily destroyed by the liver as naturally occurring œstrogens, it exerts a more prolonged action. It has a potent action when administered per os, losing only about 50% of its activity by this route.

While abortion has been induced with very large doses of diethylstilbœstrol, there is no danger of this when the therapeutic doses are used. Attempts to induce therapeutic abortion in cattle with diethylstilbœstrol have been reported. Certainly this is not a wise procedure since it is a difficult, or perhaps in some

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cases impossible, thing to accomplish; and in this species abortion is more readily effected in the early months of pregnancy simply by expressing the corpus luteum.

Hexœstrol is another synthetic œstrogen that should be mentioned. It is a substance similar to diethylstilbœstrol, having very similar properties, and is said to be less toxic but equally effective. It is not as widely used as diethystilbœstrol, however.

Posterior Pituitary Injection is used chiefly to overcome uterine inertia at the time of parturition. It is important to remember that a careful examination should precede its use in this condition to be sure that there is no mechanical impediment to passage of the fetus because, in this event, the powerful uterine contractions induced may result in rupture of the uterine wall.

Attempts have been made to expel retained placenta with pituitrin. It does not appear to be very effective in this respect. Rogers states that, in his experience, better results are obtained if the injection is made immediately at the time of calving, delay of only a half-hour reducing the action.

The male sex hormone testosterone has not been very widely used in veterinary therapeutics to date. Beckman has used it with success in the treatment of mammary tumours of bitches, reporting complete absorption of the tumour in some cases and the reduction in size and drying up of discharging sinuses in others.

The introduction of 225 mg. testosterone pellets beneath the skin of racing geldings is reported to increase the speed and endurance of some of the animals so treated.

It has been used successfully in the treatment of impotence in the male dog. A suggested dose of testosterone propionate of 10 mgm. daily for three successive days for dogs under 35 lb. wt. and 25 mgm. the first day followed by 10 mgm. daily on the next succeeding two days has been given.

Since this drug induces the descent of the testicle in immature Macacus rheseus monkeys (normally cryptorchid until puberty), its use in this condition has been suggested.

There is some indication that testosterone may reduce the enlargement of the prostate gland in benign cases.

NEWS

THE Army Veterinary Corps is inspecting daily (as of June 10) between 23,000,000 and 25,000,000 pounds of meat, meat food and dairy products. The Veterinary Corps includes 2,150 officers and about 5,000 technicians, the larger percentage of whom serve as inspectors.

* *Bulletin of U.S. Army Medical Department*, Vol. 14, No. 2, 166, August, 1945.

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